Evaluation of tris-hydroxymethylaminomethane on reversing coagulation abnormalities caused by acidosis in pigs*

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Objective: **To investigate the effect of tris-hydroxymethylaminomethane (THAM) pH neutralization on reversing coagulation abnormalities caused by acidosis.**

Design: **Random and controlled study.**

Setting: **Animal research facility and laboratory.**

Subjects: **Yorkshire swine (n = 18).**

Interventions: **Acidosis was induced in 12 pigs by infusing 0.2 M hydrochloric acid (HCl). When the target pH of 7.1 was achieved, the pigs were infused with either 0.3 M THAM to achieve pH of 7.4 (intervention group) or an equal volume of lactated Ringer's solution (acid control group).**

Measurements and Main Results: **Blood samples were taken at baseline, 15 mins after reaching pH of 7.1, and 15 mins after THAM pH neutralization. Coagulation function was assessed by thrombin generation, prothrombin time, activated partial thromboplastin time, activated clotting time, and thromboelastography (maximum clot formation time [R+K], clotting rapidity [α], and clot strength [maximum amplitude]). An additional six pigs (sham group) were infused with THAM, and an equal volume of fluid as the 12 coagulopathic pigs was given to assess effects of THAM and hemodilution. Comparisons were made using a mixed model analysis of variance. No change in any indexes of coagulation was observed in sham pigs. Compared with baseline, acidosis of pH 7.1 decreased base excess from 6.6 ± 0.5 mM to −12.4 ± 0.5 mM; reduced fibrinogen levels to 72% ± 2%, platelet counts to 53% ± 3%, thrombin generation to 58% ± 4%, α to 84% ± 2%, and maximum amplitude to 75% ± 3%; and prolonged prothrombin time to 113% ± 2%, partial thromboplastin time to 122% ± 4%, activated clotting time to 124% ± 3%, and R + K to 119% ± 3% (all $p < .05$). THAM infusion corrected pH to 7.40 ± 0.02 and base excess to 2.6 ± 0.9 mM ($p < .05$). However, there were no differences in thrombin generation, prothrombin time, partial thromboplastin time, activated clotting time, R+K, α, or maximum amplitude between the groups with or without pH correction.**

Conclusions: **Acidosis impaired coagulation by depleting clotting factors, inhibiting thrombin generation, and affecting clot strength and stability. THAM corrected acid-base deficit but did not acutely reverse the coagulation abnormalities in the model. (Crit Care Med 2007; 35:1568–1574)**

KEY WORDS: trauma; fibrinogen; platelets; thromboelastography; thrombin generation

Clinical acidosis (pH <7.25) is commonly seen in critically ill patients with metabolic derangements and trauma patients with massive blood loss and after standard resuscitation practices. Similar to metabolic derangements in critically ill patients, trauma and blood loss cause a decrease in oxygen delivery and a switch to anaerobic metabolism. Lactate production from anaerobic metabolism, if not cleared effectively, leads to metabolic acidosis. In addition, massive transfusion of blood products, which are often acidic with citrate added as an anticoagulant, contributes to the perpetuation of acidosis. Increased severity of clinical acidosis is found to be associated with increased mortality in patients (1–7). Detrimental effects from acidosis include impaired myocardial contractility, decreased cardiac output, central nervous system dysfunction, and multiple organ failure that contribute to the increased mortality (1–7). Another major consequence of acidosis is disruption of the coagulation process, although little is known about underlying mechanisms. Significantly prolonged prothrombin time (PT) and partial activated thromboplastin time (aPTT) and decreased coagulation factor levels have been commonly observed in acidotic trauma patients (2, 5, 8). In trauma patients with injury severity scores >25, Cosgriff et al. (2) reported that the probability of developing coagulopathy in response to acidosis (pH <7.1) alone was 58%. When combined with hypothermia (<34°C) and low systolic pressure, the probability rose to 98% (2). Thus, correcting acidosis-induced coagulopathy is an important strategy to reduce mortality in patients with trauma and critical illness.

Sodium bicarbonate has been used in treating acidosis for decades. However, it is increasingly clear that its administration is harmful in certain clinical settings (9–13). Since the alkaline effects of sodium bicarbonate come from CO_2 generation ($Na^+ + HCO_3^- + H^+ \rightarrow Na^+ + H_2O + CO_2$), the efficacy of bicarbonate pH neutralization is highly dependent on the host's ability to eliminate CO_2. In patients with compromised pulmonary function or tissue perfusion (such as following hemorrhage shock), bicarbonate administration may cause paradoxic worsening of acidosis. In contrast, tris-

*See also p. 1627.

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hydroxymethylaminomethane (THAM), a biologically inert amino-alcohol of low toxicity, does not generate CO_2 when used for pH neutralization. It can either combine with a proton to form $THAM^+$ ($THAM + H^+ \rightarrow THAM^+$), which is then excreted by the kidney, or combine with CO_2 to form bicarbonate to raise base excess ($THAM + H_2O + CO_2 \rightarrow THAM^+ + HCO_3^-$). Its pKa of 7.8 also makes THAM an effective buffer in the physiologic range of blood pH. In addition, unlike bicarbonate, THAM can penetrate cells and is an effective intracellular pH buffer. Thus, THAM possesses the capacity to correct intracellular, respiratory, and metabolic acidosis. With regard to coagulation, however, little is known about the effects of THAM on the coagulation process. To our knowledge, a comprehensive assessment of THAM effects on the coagulation process is not available.

Using a swine model with acidosis induced by 0.2 M HCl infusion, we reported alterations in the coagulation process following acidosis insult, including fibrinogen level depletion, thrombin generation impairment, and clotting time prolongation (14). In a search for effective interventions to correct acidosis-associated coagulopathy, we tested the efficacy of bicarbonate pH neutralization on coagulation function (15). Our data showed that although bicarbonate successfully corrected acidosis, there was no immediate improvement in coagulation function and fibrinogen levels (15). Since THAM possesses the unique capacity of correcting intracellular acidosis, respiratory acidosis, and metabolic acidosis, the present study was designed to test THAM buffer in restoring normal coagulation function, using the same animal model as previously described (14, 15). We hypothesized that THAM pH neutralization rapidly corrects coagulation abnormalities caused by acidosis. Changes in coagulation substrates, thrombin generation kinetics, and clotting function following THAM pH neutralization were compared with those without pH neutralization. In addition, we assessed the effects of THAM and hemodilution on the coagulation process in normal pigs.

MATERIALS AND METHODS

This study was reviewed and approved by the Institutional Animal Care and Use Committee. In conducting the research described here, we adhered to the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Eighteen crossbred Yorkshire swine (33.9 ± 0.8 kg) obtained from a local vendor were used in this study. After an overnight fast, animals were preanesthetized with glycopyrrolate (0.1 mg/kg), tiletamine hydrochloride (4 mg/kg), and zolazepam hydrochloride (4 mg/kg). Surgical anesthesia was induced with 5% isoflurane in 100% oxygen by mask and then maintained using 1% to 3% isoflurane after intubation. The right femoral artery and the right external jugular vein were cannulated for blood sampling and fluid infusion, respectively. Arterial blood pH was monitored continuously using intra-arterial sensors (Paratrend 7-Trendcare system, Diametrics Medical, Roseville, MN) placed via a 20-gauge carotid artery cannula. Arterial blood pressure and heart rate were monitored using an *ex vivo* pressure transducer connected to the same cannula during the study. On the completion of catheter cannulation, all animals underwent the following study protocol consisting of phase I (acidosis induction) and phase II (THAM treatment or control).

Phase I—Acidosis Induction. After baseline (T0) blood samples were taken for biochemical and coagulation analyses, acidosis was induced in 12 pigs by infusion of 0.2 M HCl in lactated Ringer's (LR) solution, following the procedures we described previously, with modification in infusion rates (14). Specifically, the 0.2 M HCl infusion started at 0.07 mL/kg/min for the initial 60-mL solution, followed by an increase to 0.14 mL/kg/min to reach pH 7.3, another increase in rate to 0.21 mL/kg/min to reach pH 7.25, and a final increase to 0.28 mL/kg/min to reach pH 7.1. Solutions of 0.2 M HCl were infused through a catheter inserted in the right jugular vein. The tip of the catheter was inserted in the vena cava to minimize any possible local damage from the infusion. This infusion procedure has been successfully used in our laboratory (14, 15). After 15 mins of stabilization, blood samples (T1) were taken for biochemical and coagulation analyses.

Phase II—pH Neutralization by THAM. In phase II, the 12 pigs were randomly divided into the pH neutralization group (Acid-THAM group, n = 6) or non-pH neutralization control group (Acid group, n = 6). In the Acid-THAM group, animals were infused with 0.3 M THAM at the rate of 0.28 mL/kg/min to neutralize arterial pH from 7.1 to 7.4. When the target pH of 7.4 was achieved and stable for 15 mins, blood samples were taken in the group (T2). In the Acid group, pigs were infused with LR solution at the same rate and duration as 0.3 M THAM infusion performed in the Acid-THAM group before T2 samples were taken.

Sham Experiment. To assess the effects of THAM buffer on coagulation in normal pigs, six additional animals (from the same vendor and with similar body weights of 32.6 ± 1.1 kg) were used in the study. In these pigs, LR was infused at the same rate and duration to mimic the infusion pattern of 0.2 M HCl fluid as in the 12 animals described in phase I. The LR infusion during this period was used to assess any possible hemodilutional effects in the study. Afterward, 0.3 M THAM was infused at the same rate and duration as the THAM infusion in the Acid-THAM group in phase II. The THAM infusion during this period was used to assess possible effects of THAM on coagulation function in normal pigs. This group was referred to as the THAM control group.

Sample Collection. To minimize coagulation effects from shear-induced platelet activation, all blood samples were collected by inserting a 25-mm single-use catheter made from Tygon tubing (Saint-Gobain Performance Plastics, Akron, OH) into the self-sealing port of the femoral catheter introducer. Blood was gently withdrawn from the catheter, and the first 3 mL of blood withdrawn was discarded at each sampling time. Heparin was not used in this study.

Analyses. Hematocrit and platelet count were measured from citrated blood using an ABX Pentra 120 hematology analyzer (ABX Diagnostics, Irvine, CA). Blood chemistries were measured using the Dimension Clinical Chemistry System (Dade Behring, Newark, DE). PT, PTT, and fibrinogen concentration were measured from citrated plasma using the BCS Coagulation System (Dade Behring, Deerfield, IL). Activated clotting time (ACT) was determined in fresh whole blood using Hemochron (HRFTCA 510 Hemochron, International Technique, Edison, NJ).

The coagulation profiles were determined for fresh whole blood with pig thromboplastin using thromboelastography (TEG) (TEG 5000 Hemostasis Analyzer, Haemoscope Corp, Niles, IL) as previously described (16). In the TEG measurements, R time is the latency time for initial clot formation; K time is the duration from initial detectable clot formation to maximum clot formation; R+K is the time for maximum clot formation; angle (α) measures the rapidity of fibrin buildup and cross-linking; and maximum amplitude (MA) represents maximum strength (mm) or stiffness of the clot.

By catalyzing fibrinogen conversion to fibrin, thrombin plays a central role in the coagulation process. Thrombin is generated from prothrombin via initiation and propagation phases. To investigate how acidosis and pH neutralization affect thrombin generation and if they could explain changes in fibrinogen levels, at least in part, we quantified thrombin generation kinetics at baseline, following acidosis induction, and at THAM pH neutralization. Specifically, thrombin generation was assessed by thrombin-antithrombin III (TAT) complex from fresh whole blood samples, following the procedure described by Rand et al (17). Briefly, fresh whole blood samples were aliquoted into 12 tubes, and a "quench" solution (50 mM EDTA and 10 mM benzamidine in HEPES-buffered saline) was added to each of

the aliquots at different time intervals to stop clot formation. The time intervals used were 1 min, 1.5 mins, 2 mins, 2.5 mins, 3 mins, 4 mins, 5 mins, 6 mins, 7 mins, 10 mins, 15 mins, and 20 mins. The quenched samples were centrifuged and supernatants were collected for TAT concentration measurement using commercially available enzyme-linked immunosorbent assay kits (Enzygnost TAT, Dade Behring, Deerfield, IL). The TAT concentrations from the supernatant samples reflect thrombin content generated from fresh whole blood samples before quench time points. This technique has been used to assess thrombin generation kinetics in various studies (14, 15, 17–19).

Statistical Analysis. All results are expressed as mean ± SE. Comparisons over time in substrate concentrations, thrombin generation, and clotting measurements within and between the groups were made using a mixed-model analysis of variance with SAS statistical analysis program (version 8.1, SAS Institute, Cary, NC). Statistical significance was set at the .05 level.

RESULTS

Abnormalities Caused by Acidosis (T0 to T1, Phase I). Acidosis of pH 7.1 was successfully induced in the 12 pigs over 233 ± 19 mins with an inflow of 44.7 ± 3.5 mL/kg 0.2 M HCl, and no animal died as a result of the acidosis. Mean arterial pressure decreased from 101 ± 5 to 72 ± 3 mm Hg during the period, and hematocrit decreased from 29.2% ± 0.9% to 25.0% ± 0.7%. In addition, arterial bicarbonate concentration, base excess, and Ca^{2+} decreased significantly following acidosis induction (Table 1). There were no significant changes in lactate levels.

Following acidosis induction, plasma total protein decreased from 5.0 ± 0.4 g/dL to 4.4 ± 0.3 g/dL, and serum albumin concentration decreased from 2.6 ± 0.2 g/dL to 2.3 ± 0.2 g/dL ($p < .05$). Fibrinogen concentration dropped from 144 ± 9 mg/dL to 98 ± 4 mg/dL ($p < .05$). The ratio of fibrinogen (mg/dL) to albumin (mg/dL) dropped from 6.2% ± 0.6% to 4.4% ± 0.5% ($p < .05$), and the ratio of fibrinogen (mg/dL) to total protein (mg/dL) dropped from 3.1% ± 0.2% to 2.2% ± 0.1% ($p < .05$), indicating a larger drop in fibrinogen concentration compared with albumin or total protein by acidosis. Platelet count decreased from 358 ± 28 $10^3/\mu L$ to 185 ± 13 $10^3/\mu L$ ($p < .05$). Clotting times of PT, PTT, and ACT were significantly prolonged by acidosis (Table 2). In TEG measurements, K time was prolonged from 1.15 ± 0.06 mins to 2.11 ± 0.31 mins ($p < .05$), but the initial clotting time R remained unchanged (2.96 ± 0.25 mins at baseline and 3.05 ± 0.26 mins at T1). The sum of R and K (maximum clot formation time) was prolonged by acidosis (Table 2). The clotting rapidity (α) and clot strength (MA) were decreased by acidosis (Table 2). A representative example of TEG profile changes by acidosis is shown in Figure 1.

Changes in thrombin generation kinetics by acidosis are shown in Figure 2*A*. In baseline blood samples, thrombin generation was 4438 ± 412 μg/L and 6182 ± 293 μg/L at 5-min and 10-min quench times, respectively. Thrombin generation remained unchanged after the 10-min quench time. At 15 mins after acidosis induction (T1), thrombin generation was 1500 ± 303 μg/L and 3338 ± 330 μg/L at 5-min and 10-min quench times, respectively, and remained unchanged after the 10-min quench time (all $p < .05$, compared with baseline values). Thus, acidosis inhibited thrombin generation by about 50% inhibition. However, the onset of thrombin generation (the initiation phase) after acidosis remained unchanged from baseline values in both groups.

Effects of THAM pH Neutralization (T1 to T2, Phase II). THAM infusion in the Acid-THAM group (24 ± 4 mL/kg) raised the arterial pH to 7.4 (T2) in 50 ± 7 mins. In addition, the deficit in base excess reversed from −12.5 ± 0.6 mM to 3.0 ± 0.6 mM ($p < .05$) but was still lower than baseline levels (Table 1). Bicarbonate concentrations were elevated from 16.1 ± 0.6 mM at T1 to 25.8 ± 1.5 mM ($p < 0.05$) but were still below baseline values (Table 1). In the Acid group, LR infusion (25 ± 4 mL/kg) during phase II did not change the arterial pH (7.11 ± 0.1 at T1 and 7.11 ± 0.1 at T2). In the Acid group (pH uncorrected), although bicarbonate concentration and base excess were increased from the values at T1, bicarbonate concentration was still below baseline values and base excess remained negative at the end of the study (Table 1).

There were no changes in hematocrit, plasma total protein, or albumin levels during phase II in either group. THAM infusion did not change fibrinogen concentration (98 ± 4 mg/dL at T1 and 94 ± 5 mg/dL at T2) or platelet counts (185 ± 13 $10^3/\mu L$ at T1 and 160 ± 24 $10^3/\mu L$ at T2) significantly. The ratios of fibrinogen to albumin and fibrinogen to total pro-

Table 1. Changes in hemodynamics following acidosis and pH neutralization

	Baseline (T0)	Acidosis Induction (T1)	Acidosis Uncorrected (T2)	Acidosis Corrected (T2)
pH	7.40 ± 0.01	7.11 ± 0.01[a]	7.10 ± 0.02[a]	7.40 ± 0.02[b]
BE, mM	6.6 ± 0.5	−12.4 ± 0.5[a]	−8.3 ± 0.4[a,b]	2.9 ± 0.7[a,b]
Bicarbonate, mM	31.7 ± 0.5	16.2 ± 0.6[a]	19.1 ± 0.7[a,b]	25.8 ± 1.5[a,b]
Lactate, mM	1.8 ± 0.3	1.3 ± 0.4	1.3 ± 0.4	1.4 ± 0.4
Ca^{2+}, mg/dL	10.0 ± 0.0	9.5 ± 0.0[a]	9.5 ± 0.0	9.5 ± 0.1

T0, baseline; T1, 15 mins after arterial pH reached 7.1 by infusion of 0.2 M HCl solution; T2, 15 mins after arterial pH reached 7.4 by Tris-hydroxymethylaminomethane (THAM) infusion in the Acid-THAM group (acidosis corrected) or pH remained 7.1 in the Acid group (acidosis uncorrected); BE, base excess.

[a] $p < .05$ compared with T0 values; [b] $p < .05$ compared with T1 values. Values are mean ± SE.

Table 2. Changes in clotting times following acidosis induction and pH neutralization

	Baseline (T0)	Acidosis Induction (T1)	Acidosis Uncorrected (T2)	Acidosis Corrected (T2)
PT, secs	10 ± 0	12 ± 0[a]	12 ± 0[a]	12 ± 0[a]
aPTT, secs	16 ± 0	20 ± 1[a]	19 ± 1[a]	20 ± 1[a]
ACT, secs	106 ± 2	130 ± 6[a]	134 ± 4[a]	134 ± 4[a]
Maximum clot formation time, mins	4.1 ± 0.3	5.2 ± 0.3[a]	5.1 ± 0.4[a]	5.1 ± 0.3[a]
Clot rapidity, α	75.5 ± 1.1	62.9 ± 2.4[a]	62.8 ± 1.9[a]	64.3 ± 1.4[a]
Clot strength, MA, mm	72.3 ± 1.4	49.9 ± 3.5[a]	52.0 ± 3.7[a]	60.0 ± 1.9[a]

T0, baseline; T1, 15 mins after arterial pH reached 7.1 by infusion of 0.2 M HCl solution; T2, 15 mins after arterial pH reached 7.4 by Tris-hydroxymethylaminomethane (THAM) infusion in the Acid-THAM group (acidosis corrected) or pH remained 7.1 in the Acid group (acidosis uncorrected); PT, prothrombin time; aPTT, activated partial thromboplastin time; ACT, activated clotting time; R+K; MA, maximum amplitude.

[a] $p < .05$ compared with T0 values. Values are mean ± SE.

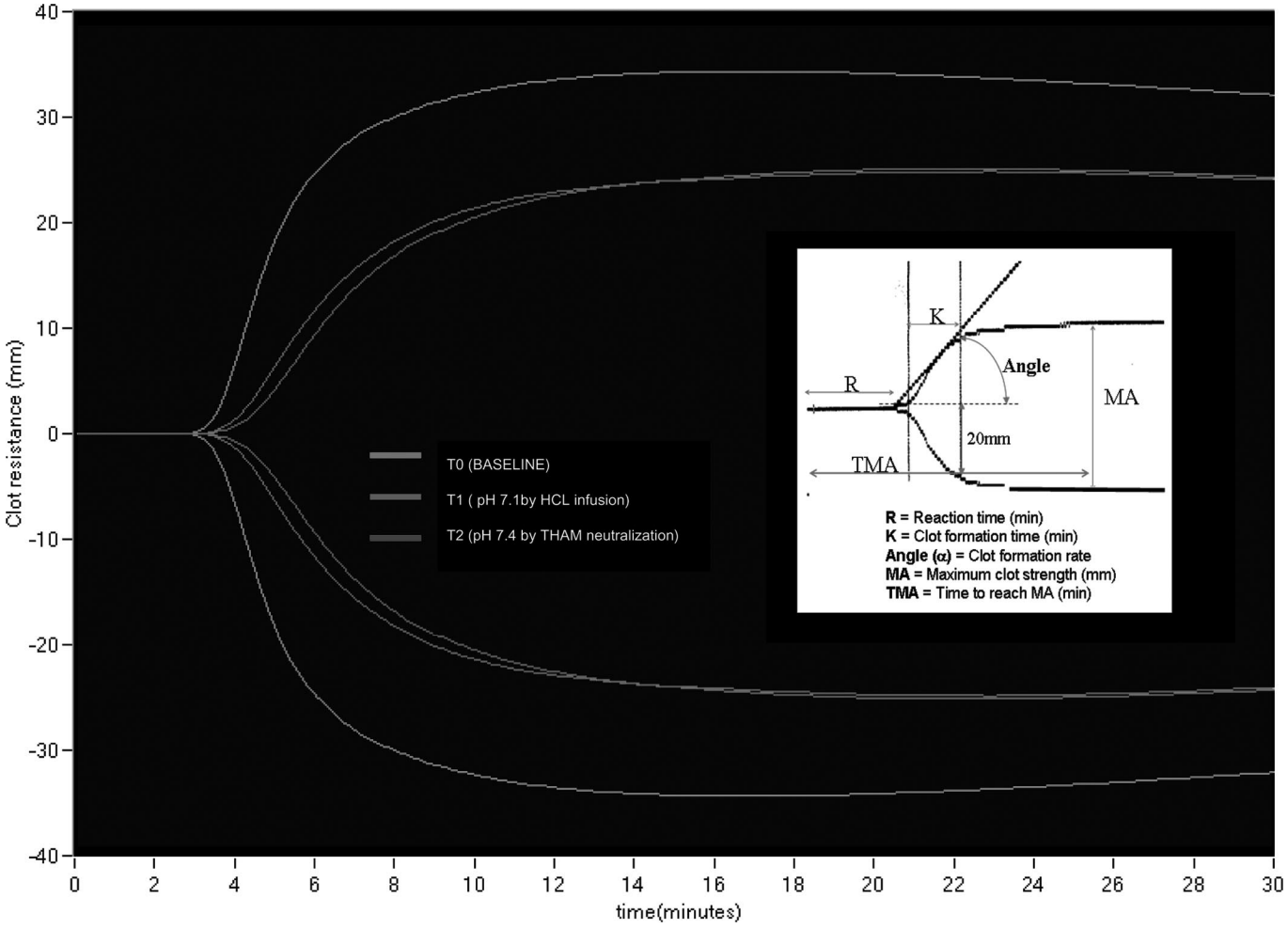

Figure 1. Thromboelastographic changes following acidosis induction and tris-hydroxymethylaminomethane (*THAM*) pH neutralization. *T0*, baseline; *T1*, 15 mins after arterial pH reached 7.1 by infusion of 0.2 M hydrochloric acid (*HCl*) solution; *T2*, 15 mins after arterial pH reached 7.4 by THAM infusion in the Acid-THAM group (acidosis corrected).

tein remained unchanged from T1 to T2. Similarly, THAM neutralization of arterial pH did not affect coagulation function. PT, aPTT, ACT, clotting rapidity (α), and clot strength (MA) remained at impaired levels in the Acid-THAM group in phase II (Table 2 and Fig. 1). During phase II (from T1 to T2), there were no differences between the Acid and Acid-THAM groups in fibrinogen concentration, platelet counts, or coagulation measurements of PT, aPTT, ACT, α, MA, or R+K (Table 2 and Fig. 1).

Thrombin generation kinetics with or without THAM pH neutralization are shown in Figure 2*B*. Following THAM pH neutralization (T2), thrombin generation was 4250 ± 601 μg/L and 6425 ± 563 μg/L at 5-min and 10 min-quench times, respectively, and remained unchanged after the 10-min quench time. In the Acid group (without pH neutralization), thrombin generation was 4437 ± 332 μg/L and 6582 ± 359 μg/L at 5-min and 10-min quench times, respectively, and remained unchanged after the 10-min quench time. These values were similar to those at baseline (4438 ± 412 μg/L and 6182 ± 293 μg/L at 5-min and 10-min quench times, respectively). Thus, thrombin generation in both groups returned to the baseline values, irrespective of pH neutralization. There were no changes in the onset of thrombin generation in either group.

Effects of THAM in Normal Pigs (THAM Control Group). In the THAM control group, LR was infused at 49 ± 4 mL/kg in 232 ± 19 mins in phase I to mimic the volume load in the acidosis-induced pigs. During the LR infusion, hematocrit was decreased from baseline values of 30.3% ± 1.0% to 26.8% ± 0.8% ($p < .05$). Total protein content decreased from baseline values of 5.1 ± 0.1 g/dL to 4.5 ± 0.2 g/dL ($p < .05$), and albumin levels decreased from baseline values of 2.8 ± 0.1 g/dL to 2.4 ± 0.1 g/dL ($p < .05$). The LR infusion did not significantly change fibrinogen concentration (122.9 ± 3.3 mg/dL at T0 and 126.3 ± 5.8 mg/dL at T1) or platelet count (342.5 ± 28.2 10^3/mL at T0 and 315.0 ± 36.4 10^3/mL at T1). Similarly, there were no changes in R+K (4.0 ± 0.5 mins at T0 and 4.3 ± 0.4 mins at T1), clotting rapidity α (74.3° ± 0.3° at T0 and 74.5° ± 0.3° at T1), clot strength MA (70.8 ± 0.8 mm at T0 and 69.9 ± 0.7 mm at T1), PT (10.2 ± 0.2 secs at T0 and 9.8 ± 0.1 secs at T1), aPTT (16.1 ± 0.1 secs at T0 and 16.6 ± 0.3 secs at T1), or ACT (103 ± 6 secs at T0 and 100 ± 2 secs at T1). In addition, there were no differences in thrombin generation kinetics in this group between T1 and T0 (data not shown). Apparently, the volume dilution involved in acidosis induction had minimal effects on coagulation. Thus, the observed coagulation changes during acidosis induction (phase I) were primarily caused by acidosis.

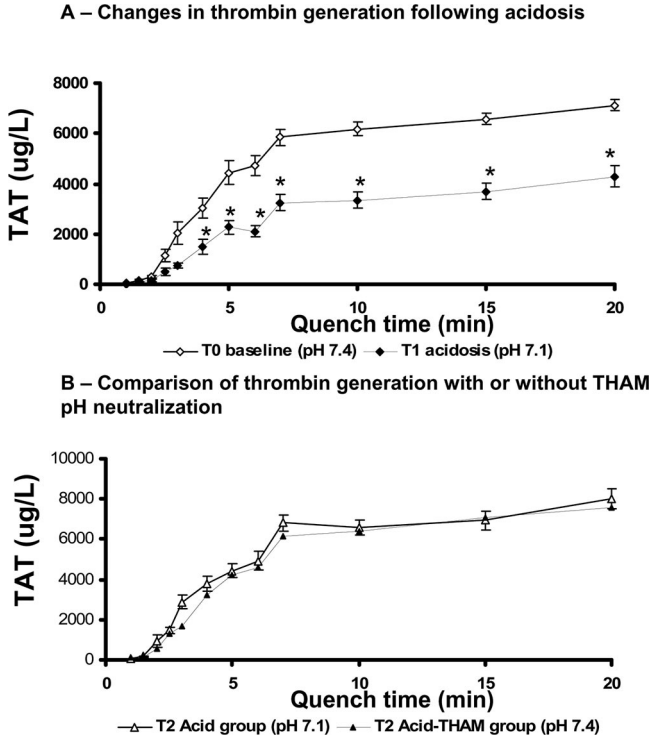

Figure 2. Changes in thrombin generation kinetics following acidosis (*A*) and with or without tris-hydroxymethylaminomethane (*THAM*) pH neutralization (*B*). *T0*, baseline; *T1*, 15 mins after arterial pH reached 7.1 by infusion of 0.2 M hydrochloric acid solution; *T2*, 15 mins after arterial pH reached 7.4 by THAM infusion in the Acid-THAM group (acidosis corrected) or pH remained 7.1 in the Acid group (acidosis uncorrected). *$p < .05$ compared with corresponding baseline values. *TAT*, thrombin-antithrombin III complex.

THAM infusion (25 ± 4 mL/kg in 50 ± 4 mins) in the THAM control group during phase II did not cause significant changes in hematocrit, total protein content, or albumin levels. There were no significant changes in fibrinogen concentration (126.3 ± 5.8 mg/dL at T1 and 121 ± 6 mg/dL at T2) or platelet count (315.0 ± 36.4 10^3/mL at T1 and 276.3 ± 32.5 10^3/mL at T2). Similarly, there were no changes in R+K (4.3 ± 0.4 mins at T1 and 4.6 ± 0.4 mins at T2), clotting rapidity α (74.5° ± 0.3° at T1 and 74.2° ± 0.4° at T2), clot strength MA (69.9 ± 0.7 mm at T1 and 69.8 ± 0.9 mm at T2), PT (9.9 ± 0.1 secs at T1 and 10.0 ± 0.1 secs at T1), PTT (16.6 ± 0.3 secs at T1 and 16.9 ± 0.3 secs at T2), or ACT (100 ± 3 secs at T1 and 97 ± 3 secs at T2). In addition, there were no differences in thrombin generation kinetics from T1 to T2 in this group (data not shown). Thus, THAM buffer in this study did not significantly change any coagulation indexes measured in normal pigs.

DISCUSSION

An altered coagulation process is commonly observed in acidotic patients (2–5, 8, 9). Using a swine model with acidosis induced by HCl infusion, we observed similar detrimental effects from acidosis on the coagulation process in this study. Acidosis caused a 30% decrease in fibrinogen level, 50% decrease in platelet counts, and 50% acute inhibition in thrombin generation. Consequently, clotting rapidity was decreased, clot strength impaired, and clotting times prolonged. The infusion of 0.3 M THAM buffer successfully corrected acidosis. However, alterations in coagulation by acidosis remained unchanged at 15 mins after arterial pH was corrected to 7.4 by THAM. Thus, coagulation impairment by acidosis was not immediately reversible by pH neutralization alone. Similarly, Abdel-Rassoul et al. (20) reported that correction of respiratory acidosis by TRIS buffer failed to reduce blood loss in dogs. Those observations emphasize the complexity and challenges in restoring coagulation function after development of acidosis.

Normal hemostasis requires sufficient clotting substrates and enzyme activities for the coagulation process. Hemodilution may affect the clotting process by diluting clotting factors and enzymes. In this study, acidosis of 7.1 was induced by infusing 1.5 L of 0.2 M HCl over 4 hrs in 12 experimental animals in phase I. This fluid infusion decreased hematocrit, total protein, and albumin about 5% to 10%, decreased fibrinogen about 30%, and decreased platelet counts about 50%. These decreases were accompanied by impairment in clotting time, clot strength, and stability. Our findings of acidosis effects on coagulation have been similarly observed *in vitro* by other investigators. By adding either lactic acid or hydrochloric acid to human blood samples to pH between 7.4 and 6.8, Engstrom et al. (21, 22) showed impairments in clotting strength and increases in clotting time. To clarify possible hemodilutional effects in the present study, a sham group infused with an equal volume of LR was included in this study. There were 5% to 10% decreases in hematocrit, total protein, and albumin, but changes in fibrinogen concentration, platelet counts, and coagulation were not observed in the sham group throughout the study. A similar lack of these changes from up to 30% hemodilution *in vitro* with LR solution has been observed by our colleagues AV Delgado and BS Kheirabady (personal communication, July 9–14, 2006). Thus, the decreases in fibrinogen and platelets observed in the acidosis-induced pigs were primarily due to acidosis, not hemodilution.

Acidosis of pH 7.1 caused a 50% decrease in platelet counts in this study. The mechanisms leading to the decrease remain unclear. Djaldetti et al. (23) showed that when pH dropped below 7.4, platelet internal structure and shape changed to become spheres deprived of pseudopodia, suggesting that the structural changes might possibly lead to accelerated removal of platelet from circulation. In this study, there was also a 30% decrease in fibrinogen levels following acidosis induction. This decrease could have been due to inhibited synthesis, accelerated degradation, or both. Since fibrinogen synthesis under normal circumstance is about 2% to 4% per hour (24, 25), inhibition in synthesis is not likely the important contributor to the 30% decrease. Rather, it is likely due to an accelerated degradation. Regardless of the underlying mechanism contributing to the loss of fibrinogen, it is clear from this study that the loss of fibrinogen was irreversible, at least acutely because fibrinogen levels remained at depleted levels after pH was neutralized to 7.4 by

THAM. Apparently, pH neutralization might prevent further deterioration, but it cannot compensate immediately for the loss of fibrinogen occurred.

Catalyzing the conversion of fibrinogen to fibrin clot, thrombin plays an important role in the coagulation process. Thrombin is generated from precursor prothrombin through the initiation phase and the propagation phase. In this study, as much as 50% inhibition in the propagation phase of thrombin generation was observed 15 mins after arterial pH reached 7.1, with no changes in the initiation phase of thrombin generation. When the arterial pH was neutralized to 7.4 by THAM, the inhibition in thrombin generation was not observed and thrombin generation kinetics returned to baseline levels. Interestingly, in the pH uncorrected group, we observed an unexpected, similar recovery of thrombin generation at the end of the study (about 1 hr after arterial pH reached 7.1), indicating a possible self-compensatory recovery from acidosis in thrombin generation. The fact that the recovery of thrombin generation kinetics shown in both groups was not accompanied by a recovery in coagulation function in either group suggests that replenishing coagulation substrates might be more essential to restore normal coagulation function. Thus, our data of changes in clotting factor availability and thrombin generation kinetics confirm the importance of supplementing coagulation factors with acidosis associated coagulopathy.

Although Moon et al. (26) reported that THAM infusion did not change platelet count, PT, or aPTT in normal dogs, a comprehensive assessment of the effects of THAM on coagulation profiles under normal circumstances has not been done. In our study, sham pigs were used to evaluate the effects of THAM on coagulation under normal circumstances. We found that THAM infusion did not cause any significant changes in fibrinogen levels, platelet count, or thrombin generation kinetics. Neither were there changes in PT, PTT, ACT, clotting rapidity, or clot strength. Thus, it is reasonable to consider that THAM by itself has minimum effects on coagulation and that our observations of coagulation following THAM pH neutralization reflect primarily the consequences of a metabolic acidosis. In addition, with its minimal effects on coagulation, our data support THAM as a pH neutralization agent in clinical practice.

In this study, a comprehensive approach was used to assess the effects of acidosis and arterial pH neutralization on coagulation. Our measurements included clotting substrate availability, thrombin generation kinetics, and clotting functionality assessments of PT, PTT, ACT, and TEG. Consistent and complementary results were observed in this study. For example, the initial clotting time (R) in TEG was not changed in either group throughout the study, which coincided with unchanged initial thrombin generation in all the groups. Both clot strength (MA, affected by platelet and fibrinogen levels) and clotting rapidity (α, induced by thrombin generation) were decreased following acidosis, which agreed well with depletions in fibrinogen level, platelet counts, and inhibition in thrombin generation. Furthermore, clotting times from different measurements (PT, PTT, ACT, and R+K from TEG) were consistently prolonged by acidosis and remained prolonged after pH neutralization with THAM.

CONCLUSIONS

We investigated coagulation alterations induced by acidosis and the potential for THAM to reverse the altered coagulation function. Acidosis impaired the coagulation process by depleting some coagulation substrates and inhibiting the thrombin generation burst. As a result, clotting time was prolonged and clot strength compromised. THAM pH neutralization did not immediately reverse any of the coagulation changes, particularly the depletions in fibrinogen levels, platelet counts, and clotting times. We stress that changes in coagulation observed in the study only reflect acute changes from acidosis as an individual factor. Trauma patients often experience tissue injury, blood loss, surgery, and standard resuscitation in addition to acidosis. In addition, coagulation profiles in patients are often observed days or weeks after trauma injury or surgery. Thus, these multifactorial and timing differences are likely to cause different coagulation changes in actively bleeding trauma victims.

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